Studies on Marine Microbial fuel cell

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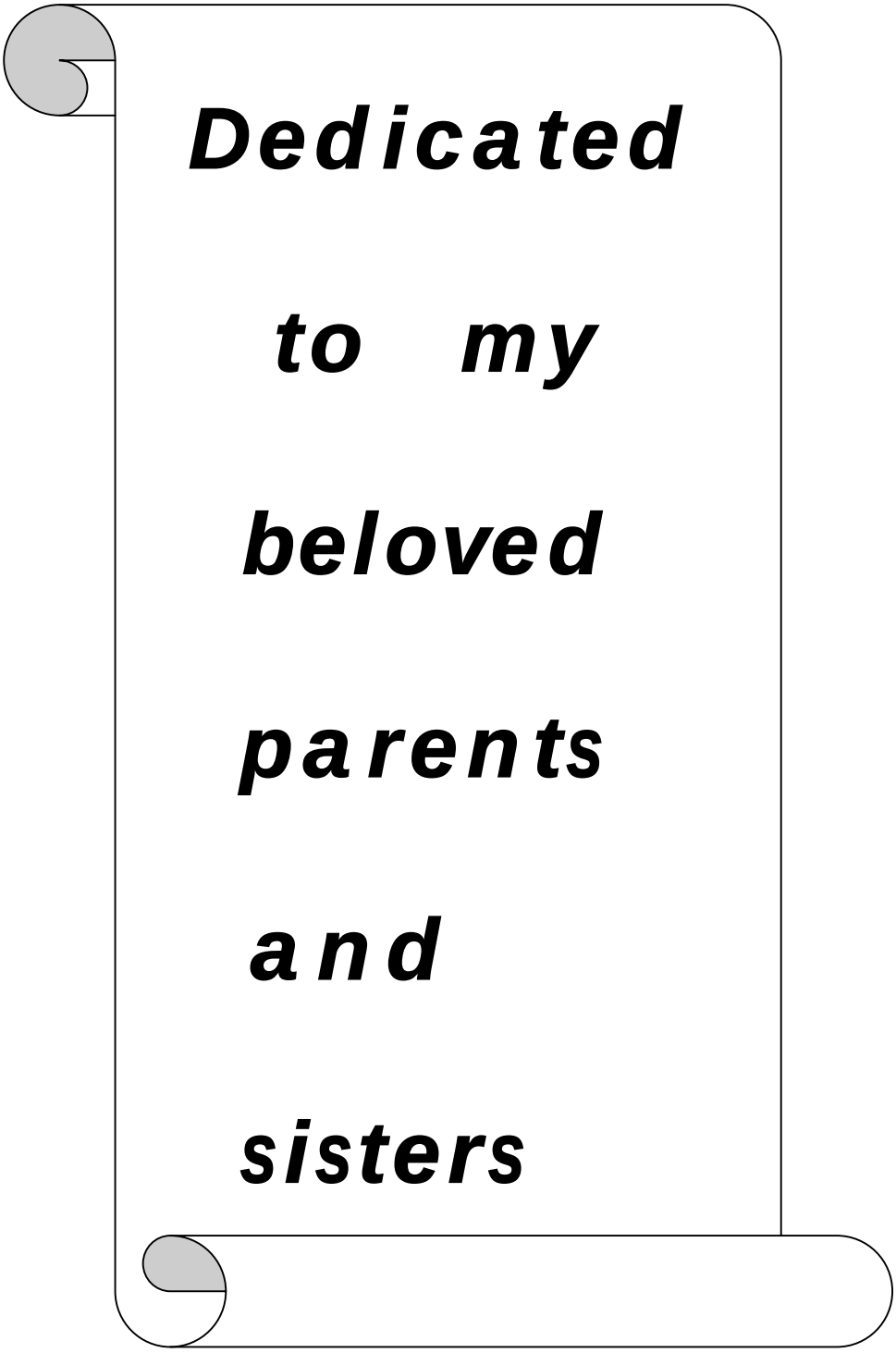
Dedicated
to my
beloved
parents
and
sisters

DEPARTMENT OF LIFESCIENCE
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CERTIFICATE

This is to certify that the thesis entitled “Studies on marine microbial fuel cell” which is being submitted by Miss. Supriya kumari, Roll No. 410LS2055, for the award of the degree of Master of Science from National Institute of Technology, Rourkela, is a record of bonafied research work, carried out by her under my supervision. The results embodied in this thesis are new and have not been submitted to any other university or institution for the award of any degree or diploma.

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DECLARATION

I hereby declare that the thesis entitled “Studies on Marine Microbial fuel cell ”, submitted to the Department of Life Science, National Institute of Technology, Rourkela for the partial fulfilment of the Master Degree in Life Science is a faithful record of bona fied and original research work carried out by me under the guidance and supervision of Dr. Surajit Das, Assisttant Professor , Department of Life Science, National Institute of Technology, Rourkela. To the best of my knowledge no part of this thesis has been submitted to any other institutes or organization for the award of M Sc. degree.

Date:~

Place:~

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This project is by far the most significant accomplishment in my life and it would not have been impossible without people who supported me and believed in my caliber.

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My heartfelt thanks to my all classmates for their moral support, help and encouragement throughout the course of this work. I take the pleasure to acknowledge the constant help and support of my friends has always been cherished.

Lastly, I acknowledge with highest sense of regards to my parents and sisters for their blessings, unwavering support, love and affection without which the parent investigation would not have been successful in any sphere of my life.

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Supriya Kumari

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ABSTRACT

The chemical process that bacteria utilize to shift electrons to their neighboring environments are assorted and not well understood. This study provides novel information that initiate to reveal correct mechanisms involved with electron transfer to microbial fuel cell (MFC) electrodes using various bacterial isolates. In the present study, effect of varied salt and carbohydrate (dextrose) concentration was studied in a novel MFC design was observe. In the optimal salt concentration, the MFC produced a maximum current of 262 µA and in optimum concentration of dextrose a maximum of 1033mV. The various combinations of bacterial isolates were also studied and found that they are much more efficient in generating high potential difference. These data have implication toward the optimization of bioremediation technologies, MFC advancement and to the basic understanding of how bacteria work together with their environments. This determines the choice and performance of specific organisms.

Key Words – MFC, Current, bioremediation, electrodes.

1. INTRODUCTION

Energy calamity in India is rising each year, as there is constant acclivity in the price of fuels and also due to depletion of fossil fuels to a larger level (Reddy *et al* 2007). The demand for an alternating fuel has erupted extensive research in discovering a potential, economical and reusable source for energy manufacture. For constructing a sustainable world we require to minimize the expenditure of fossil fuels as well as the pollution generated. These two aims can be accomplished all together by treating the waste water (From disposing waste to using it). Industrial waste, agricultural waste and household waste are ideal substrates for energy productions as they are rich in organic contents.

MFC can be best defined as a fuel cell where microbes act as catalyst in degrading the organic content to produce electricity. It is a device that straight away converts microbial metabolic or enzyme catalytic energy into electricity by using usual electrochemical technology (Allen and Bernetto, 1993). MFCs function by directly capturing the electrons generated when electrochemically dynamic bacteria degrade organic substrates. MFC have become popular as it has the capacity to produce energy in the form of electricity or hydrogen from renewable sources like industrial or household waste.

The outfitted and working advantages of MFCs are:

- It uses organic squander stuff as fuels and easily available microbes as catalysts.
- It do not require extremely synchronized division systems like the ones needed for Hydrogen Fuel Cells.
- MFCs have high alteration effectiveness as compared to Enzymatic Fuel Cells, in harvesting up to 90% of the electrons from the bacterial electron transport system.

MFCs have operational and functional advantages over the technologies currently used for generating energy from organic matter. Initially, the unswerving conversion of substrate energy to electricity enables high conversion efficiency. Second, MFCs operate efficiently at optimum and even at low, temperatures distinguish them from all present bio-energy processes. Third, an MFC does not entail gas treatment because the off-gases of MFCs are enriched in carbon dioxide

and usually have no useful energy contented. Fourth, MFCs do not need energy contribution for aeration provided the cathode is submissively aerated (Liu, 2004). Fifth, MFCs have potential for widespread application in locations lacking electrical infrastructures and also to expand the diversity of fuels we use to satisfy our energy requirements.

Fuel Cell (MFC) commonly consists of two chambers, one anaerobic anodic chamber (anode) and the other aerobic cathodic chamber (cathode). Substrate is oxidized by bacteria in the anaerobic chamber and the electrons transferred to the anode by an external electron carrier or by mediator (such as methylene blue, potassium ferric cyanide, thionine, or neutral red) or unswervingly from the bacterial respiratory enzyme to the electrode. The marine environment provides a good example of a mediator less MFC (Tendler *et al.*, 2002). But there many limitations steps of mediator-less MFC (Gil et al., 2003). They are :

(1) Fuel decomposition at the anode,

(2) Electron transport from microbial cells to anode,

(3) Resistance of the circuit,

(4) Proton transfer through the membrane, and

(5) Oxygen decrease at the cathode.

The use of microorganisms in microbial fuel cells decimates the partition of individual enzymes, thereby providing economical substrates for microbial fuel cells (Schroder et al., 2003).

The anaerobic chamber is linked to the aerobic chamber by a proton- channeling material (e.g. Salt bridge, membrane etc.) and outwardly by a wire that completes the circuit. Electrons are transferred to the cathode chamber through the external circuit, and the protons through the membrane.

Chemotrophic microbes utilize organic and other recyclable compounds, under extreme conditions. The electrons resulting from the oxidation are imparted to an electron transport chain, across appropriate electron carriers depending on the final electron acceptor molecule. In aerobic organisms, this final acceptor is oxygen which takes up the electrons and gets reduced to water. The chemiosmotic hypothesis states that electron transfer chains of bacteria are tied to the

movement of protons transversely the membrane which is in turn coupled to ATP synthesis by the proton electrochemical potential crosswise the energy transducing membrane. The bacterial cell membrane functions as an energy transducing membrane working in accordance to the chemiosmotic theory. The movement of protons towards the exterior of the membrane results in the organization of a proton electrochemical slope. The pH gradient add up to this membrane potential and outcome is the proton drive or motive force. The re-access of these protons transversely to the ATP-synthase enzyme is went with ATP production (Nicholls, 1982) . The ATP synthesized thus is used by the bacteria for their endurance (Moat et al., 2002).

The electron donor is actually estranged from the ultimate electron acceptor across the two chambers. The majority of the electrons released from the progression of oxidation are channeled to the anode. Electron transfer to the anode can be consummated by:

- Electron mediators or locomotary factors (Rabaey et al., 2004),
- straightforwardly by the cell (Bond and Lovely, 2003)
- By means of 'nanowires' (Reguera et al., 2006).

These electrons are aimed to the cathode across a peripheral circuit and for every electron conducted, a proton is elated across the membrane to the cathode for carrying out the reaction and supporting the electric current (Logan and Regan, 2006).

MFCs needing external mediators have limited practical use because chemicals used as mediators are costly and toxic to bacteria (Bond *et al.*, 2002).

However MFC technology is still elementary and there are several areas for development (Rabaey and verstsaete, 2005). Traditional MFC show low coulombic efficiencies due to ineffective electron transfer linking the microbial cells, and the anode. This ineffectiveness consequence in partial oxidation of the fuel and unsought digestion of some of the fuel carbon in to biomass.

Even if there has much work to optimize MFC forms, their physical and chemical working conditions and the choice of microorganisms (Logan, 2005). There is a increasing attention in the use of redox –active enzymes as both anodic and cathodic catalysts in micro or even nanoscale

fuel cells (Rismani et al., 2008). Electrochemical mediator dyes are classically used to increase the effectiveness of electron transfer in MFCs that utilize pure cultures of microorganisms (Kim et al., 1982).

In waste mass from farming, urban, domestic and industrialized sources, plentiful quantity of energy is laid in chiefly in the form of carbohydrates.

Microorganisms tacked together in biofilms also have electroactivity. Electroactive microbial biotic communities are also found in marine sediments, actuated mud, manure communities, and soils (Logan, 2009).

MFC technology can be used as a feeler for pollutant study and *in situ* process monitoring and control. Biological Oxygen Demand (BOD) is the sum of dissolved oxygen needed to get together with the metabolic requirements of aerobic organisms in water rich in organic matter, such as sewage. The relative association between the coulombic yield of MFCs and the concentration of assimilable organic contaminations in wastewater make MFCs possible functional as BOD sensors.

MFCs can also be adapted to produce hydrogen gas by taking away oxygen fromt the cathode and adding in a small voltage via the bio electrochemically aided microbial reactor (BEAMR) process or the biocatalyzed electrolysis process. MFC can also be used for desalinating seawater as Fresh water sources are running out.

The long-standing steadiness of the MFC depends on enforcing process control to sustain means of working parameters in a required range. Some of these parameters comprise pH, substrate charging, flow velocity and oxygen transportation into the anodic chamber.

Chemical energy can be transformed to electrical energy by pairing the biocatalytic oxidation of organic or inorganic compounds to the chemical reduction of an oxidant at the edge between the anode and cathode (Willner et al.,1998).

Fuel Cell (MFC) usually comprises of two chambers, one anaerobic chamber (anode) and the other aerobic cathodic chamber (cathode). In the anaerobic chamber, substrate is oxidized by bacteria and the electrons shifted to the anode either by an external electron transporter or mediator (such as potassium ferric cyanide, thionine, or neutral red) (Delaney *et al*., 1984; Park

and Zeikus, 2000; Rabaey *et al.*, 2004), or unswervingly from the bacterial respiratory enzyme to the electrode. This system has been semi biological, because only the anode side holds electrochemically-active microorganisms, while the cathodic chamber is maintained at abiotic condition.

However, soluble mediator confines the use of MFCs to congested systems. To solve the problems of low coulombic effictiveness while quashing the use of mediators, some investigators have used solitary species of microorganisms that use the anode directly as fiinal electron acceptor (Logan and Regan, 2006). Enzyme catalyzed fabrication of NADH (dihydro-nicotinamide adenine dinucleotide) from alcohol, lactic acid, amino acids, formate or other overflowing substrates could give the bio- shifts that activate the anodic compartment of the fuel cell (Williner et al., 1998). Theoretically, any organic or inorganic compound or a mixture can be furnished as a fuel, depicted it is oxidized by the proper organism, e.g., the reaction for glucose is (Kosaric and Velikonja ,1995) .

$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24e^- + 24\ H^{+\cdot}$

The pairing of metabolic oxidation of the initial electron donor (NADH) to reduction of the terminal electron acceptor (such as oxygen or fumarate in bacterial respiration systems) is very similar to the pairing of the electrochemical half-reaction of the reductant (electron donor) to the half-reaction of the oxidant (electron acceptor) in a fuel cell or battery system (Chang, 1981).

The process by which electricity is produced by microorganisms is shown in the figure given below (figure-1). The normal process of electron transport chain occurs in the anodic compartment.

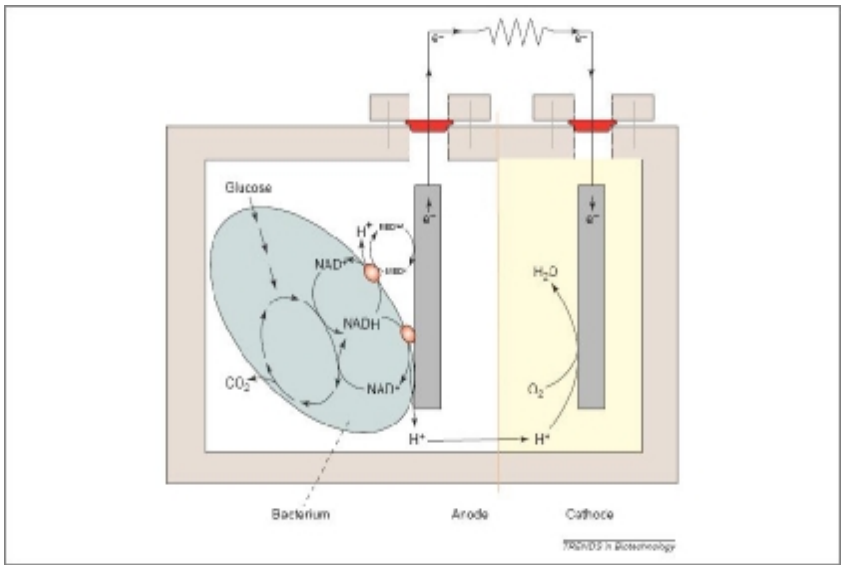

Fig. 1. Schematic representation of a microbial fuel cell – the anodic chamber comprising metabolizing biocatalysts is illustrated on the left chamber, and the cathodic chamber constitutes an open air type. Both chambers are connected outwardly by an electrical circuit and internally by a proton swap membrane.From (Rabaey and Verstraete, 2005)

Biological reducing power sources with low redox potentials, such as NADH (E_0'= - 0.32 V), reduced ferredoxin (FdH2) (E_0' = -0.42 V), or reduced flav

in adenine dinucleotide (E_0'= -0.19V), can act as reductants for fuel cells, but they are not easily transformed to electricity because the cytoplasmic membrane has to be non conductive to maintain the membrane potential absolutely needed for free energy (i.e., ATP) production (Thauer et al., 1977).

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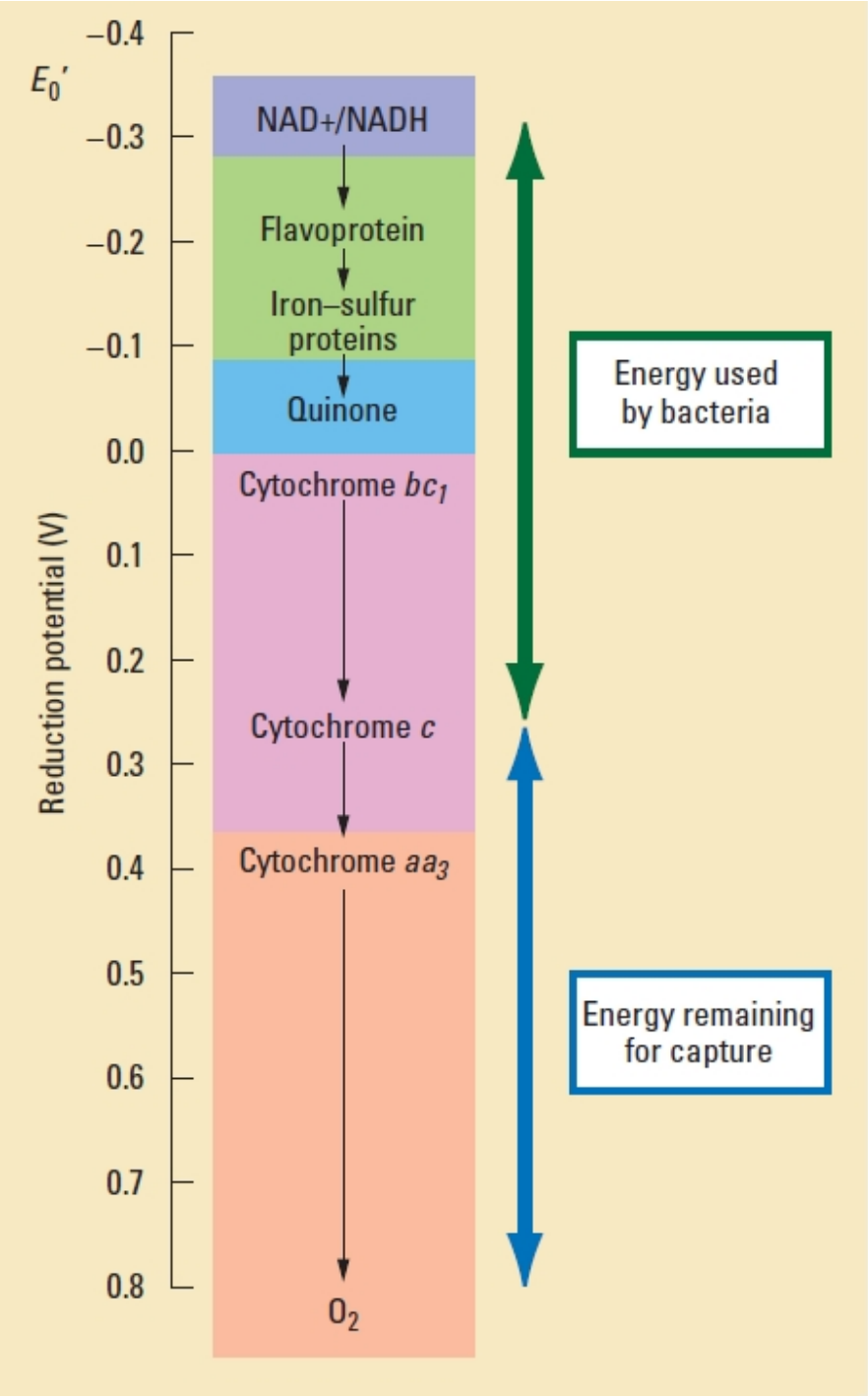

Figure-2 Electron transport chain

In the Figure -2 above it has been shown there that bacteria could gain energy from the potential between NADH (the reduced form of nicotinamide adenine dinucleotide) and cytochrome *c*

(green arrow), whereas the MFC could pick up energy from the potential between cytochrome *c* and oxygen (blue arrow). Real potentials depend on concentrations and potentials of specific enzymes and electron acceptors.

High anodic potential is wanted for increased energy production, while lower potentials can lead to electron loss via transfer to unconventional acceptors, like sulfates, or the generation of by-products like methane. This is accomplished primarily by excluding oxygen from the chamber. To keep the anodic chamber free of oxygen to retain redox potential, fermentative organisms must be selected. The cathode completes the circuit of the cell by channeling electrons to a high-potential electron acceptor.

The pH and buffering properties of the anodic chamber can be assorted to get the most out of microbial expansion , energy creation, and electric potential (Du Z et al., 2007).

MFCs microbial communities can be classified into three classes: heterotrophic cells, photoheterotrophic cells, and cells from the aqueous sediments. Heterotrophic cells include a sole identical colony of microbes whichever poised in media or in biofilms growing on electrodes. Photoheterotrophic cells consume photoheterotrophic microbes able to act as the biocatalysis of microbial metabolism in addition to photosynthetic sources. Sedimentary cells use microbial communities lodging in marine environment to generate electric potential (Rabaey et al., 2003).

The main problems presently hampering the progress of development associate to quantifiability issues: how to use MFCs on an industrialized scale while still asserting low costs, least hazardous, and maximum energy output.

2. REVIEW OF LITERATURE

Energy

Energy is outlined as the capability to try and do work. We have a tendency to use energy to do work and create all movements. After we eat, our bodies remodel the food into energy to do work. Once we run or walk or do some work, we 'burn' energy in our bodies. All the vehicles, airplane, trolleys, boats, and machinery conjointly convert energy into work. There are several sources of energy that facilitate to run the assorted machines invented by man (Amann,1996).

Energy fabrication and supply are challenging due to the exhaustion of fossil fuel .Presently worldwide energy necessities are generally dependent on fossil fuel which eventually leads to the probable diminution of inadequate fossil fuel assets (Amann, 1996; Das and Vetziroglu, 2001). Liberation of global warming gases such as CO_2 due to burning of fuels is more of concerned. Increase in requirement of energy resources and concern about climate changes are driving to hunt for unconventional energy for fossil fuels (Logan, 2004) .

The first indication which proves that microorganisms could aid an electrical signal during their metabolic processes and thus can be substitute source of energy and was innovated in the year 1911 by M.C. Potter (Potter, 1911). Microbes can be employed for the translation of chemical energy in forms of fuels (biogas, bioethanol, biohydrogen) or directly to electricity by oxidation of organic substances (Logan, 2004).

Bioenergy is one of the most significant gears to alleviate greenhouse gas discharges and reserve of fossil fuels .The call for of energy is rising incessantly, because of increasing industrial enterprises and population. The fundamental sources of this energy are petroleum, natural gas, coal, hydro and nuclear power plants (Kulkarni and Dalai, 2006).

In 2008 the yearly world primary energy expenditure was likely 11,295 million tons of oil equivalent, fossil fuels reported for 88% of the primary energy utilization, with oil (35% allocate), coal (29 %) and natural gas (24 %) as the most important fuels, while nuclear energy and hydroelectricity accounted for 5% and 6% of the total initial energy utilization, respectively (Khan et al., 2009).

Solar energy can provide an substitute energy source for MFC function. The perception of 'living solar cell' was anticipated in which the green alga *Chlamydomonas reinhardtii* produces hydrogen photosynthetically which in turn is oxidized in situ to generate current. phototrophic MFCs characterize a move toward conversion of solar energy into electrical energy either through photosynthetic microorganisms or living plants (Strik et al.,2008 and He et al., 2009).

Sources of biomass for energy

Every kind of biomass can be used to both for burning it for energy or to obtain one or other fuel from it. Some species provide better quality of fuel at lesser costs than other species. Energy from biomass is built around such species. Food crops to energy programs are under increasing scrutiny because they compete with the use of these crops as food, there by pushing up food prices and threatening the existence of subsisting individuals.They also acutely corrupt land and water masses (Abbasi and Abbasi, 2010).

A large number of plants contain hydrocarbons in significant concentration enough to become a potential source of diesel like firewood. In just the north eastern region of India , 99 species of such lactiferous (latex yielding) species have been recognized. Familiar among hydrocarbon plants are *Jatropha* (seven species) and *Euphorbia* (five species) (Kalita, 2008). The terrestrials mimosa and lantana, the *Amphibian ipomea,* and the aquatics water hyacinth, Salvinia and Pistia are examples of weed capable of generating electricity (Gajalakshmi et al., 2001).

Microbial fuel cell

Fuel cells have the ability to formulate electricity in a non-polluting manner. A microbial fuel cell (MFC) also serves in the exclusion of organic, or inorganic, waste from water supplies while producing a by-product of electricity (Roller et al., 1984; Allen and Bennetto 1993; Liu et al. 2004; Min and Logan 2004; Min et al. 2005; Bullen et al. 2006; Lane 2006; Logan et al., 2006).

Microbial energy transformation in Microbial Fuel Cell, a bioreactor in that bacteria transform the chemical energy in biomass unswervingly to electricity is a hopeful technology for renewable energy production. (Chaudhuri and Lovley, 2003; Rabaey and Verstraete, 2005). A MFC is an electrochemical system, in which living microorganisms are employed as catalysts to force the oxidation (Cohen 1931; Roller et al. 1984; Allen and Bennetto 1993; Kim et al. 1999; Bond,

Holmes et al. 2002; Rabaey et al. 2003; Liu , 2004) and/or reduction reactions (He and Angenent, 2006; Clauwaert et al. 2007; Rozendal et al. 2008).

Besides bacteria, yeast was also efficient in MFC in MFC experiments. But it was less active and giving less reducing power than bacteria (Bernetto, 1984). Microbial fuel cells household waste water systems were accessible by Habermann and Tommer,(1991). Microbial fuel cells which were operated in continuous mode, were more appropriate for sensible applications than fed batch ones. Three influent types containing carbohydrates were started. i.e. glucose medium, a plant extract and artificial wastewater. The anode reactor compartment yielded best results, when it was packed with graphite granules. In non mediated batch systems power outputs up to 479Wm^{-3} but in continuous mode the power outputs were limited to 49Wm^{-3} (Rabaey and Verstraete, 2005).

Microbial fuel cell utilizing cheap materials (non coated plain graphite electrodes) without any noxious mediators (aerated cathode and mediator less anode) was assessed under acidophilic (anode pH 5.5) conditions using anaerobic mixed consortia to count the influence of substrate lading rate on bio electricity production from anaerobic wastewater treatment at ambient temperature (28° C) . Maximum potential difference of 423 mV (1.66 mA) was read at steady working conditions. Apart from power generations, the fuel cell also established substrate elimination of 62.5%. Voltage and current started diminishing due to substrate exhaustion (COD reduction) in the anodic chamber. The study documented the advantage of both wastewater treatments and electricity production in a sole system (Venkata Mohan et al., 2007).

Microbial Fuel cells with phenol

MFC using phenol or glucose phenol mixtures as the substrate (fuel) were designed to investigate the biodegradation of phenol. In an aqueous air cathode MFC using phenol (400 mg L^{-1}) as the sole fuel, electricity was generated during the phenol degradation. Microbial fuel cells can be used straightforwardly to generate electricity from the oxidation of dissolved organic matter but optimization of MFCs will require the knowledge of factors which can improve the power output. The type of proton exchange system used can affect the system inner resistance. Power output in a MFC containing proton exchange membrane was compared using a pure culture *Geobacter metallireducens*) or a mixed culture (wastewater inoculums). Power

output with either inoculums was essentially the same , with 4071 mW m^2 for *Geobacter metallireducens* and 3871 mW m^{-2} for the waste water inoculums (Booki et al.,2005).

Microbial fuel cell containing polypyrrole coated coated carbon nanotubes

A microbial fuel cell was fabricated using polypyrrole coated carbon nanotubes amalgamated as an anode material and *Escherichia coli* as the biocatalyst. The polypyrrole carbon nanotubes indicated better electrochemical performance than that of plain carbon electrodes. The power output of the MFC increased along with the increase of the composite loading. In the absence of pyrrole carbon nanotubes exhibited a maximum power density 228m Wm^{-2}, which is much higher than those reported in the literature so far for *E. coli* using efficient electron mediators (Yongjin et al., 2008).

Mechanism of microbial fuel cell

The perfect presentation of an MFC depends on the electrochemical reactions that occur between the organic substrate at a low potential such as glucose and final electron acceptor with a high potential, such as oxygen (Rabaey and Verstraete, 2005). Those bacterial species that are incompetent of expelling electrons to the anode straighlyt a redox mediator is needed to transfer the electrons straighttly to the anode (Stirling et al., 1983; Bernetto, 1984). In mediator-less MFCs utilizing anodophiles such as *G. sulfurreducens* and *R. ferrireducens*, microbes form a biofilm on the anode surface and utilize anode as their ultimate electron acceptor in their anaerobic respiration (Hernandez and Newman, 2001).

Fermentation products such as acetate can be oxidized at low anode potential by anaerobic bacteria such as Geobacter species, which is capable of diminishing electrons from acetate in MFC conditions (Vandevivere et al., 2001). The unspecified *Desulfovibrio* strain can act as a biocatalyst to oxidize calcium lactate to electrons and protons in anaerobic artificial seawater media (Sisler, 1961). Sisler used a potassium chloride salt bridge to link the anode compartment, containing *Desulfovibrio*, with the cathodic compartment holding sterile, oxygenated seawater as the electrolyte. The cathode reduction reaction of oxygen was supposed to be abiotic. Sisler was able to produce an open-circuit cell voltage of 0.5 V and described a maximum current .in excess of one milliampere. Here the author necessitated that these biochemical fuel cells could use any number of microorganisms to constrain the catalytic reactions and that these systems could be

spread in remote areas to act as sustainable energy sources. In a 1962 publication by Sisler, he abstracts the importance of this research to the United States Navy and briefly discusses the Biological Electrical Energy Production (BEEP) project that was conducted by the Advanced Concepts Division, Bureau of Ships (Sisler ,1962). It was reported that BEEP studied many variations of biological fuel cells together with systems with diverse electrodes, yeast, algae, bacterial strains and enzymes. The current densities that resulted from these studies were observed to be between 0.10 and 10.0 A/ft2 (Sisler, 1962).

The anodic reaction in mediator less MFCs constructed with metal reducing bacteria belonging primarily to the families of *Shewanella, Rhodoferax* and *Geobacter* is similar to that in this process because the anode acts as the final electron acceptor just like the solid mineral oxides (Holmes et al., 2004). The power density was raised from 0.44mWm−2 to 91 mWm−2 (with the adapted electrode) when mediator was used, *Escherichia coli* being the biocatalyst (Park and Zeikus , 2003). However, when the pure culture of *E. coli* was substituted with sewage sludge the power density in the enhanced reactor rose to 788mWm^{-2}. This would suggest that sewage sludge unexpectedly contains efficient electrophilic organisms (Park and Zeikus , 2003).

Clostridium beijerinckii, Clostridium butyricium, Desulfotomacum reducens, Rhodobacter capsultatus, Thiobacillus ferroxidans and even the *Geovibrio* genus are all capable of use in a mediatorless fuel cell as the organisms, some of which were isolated from a fuel cell utilizing starch Wastewater (Park et al., 2001 ; Pham et al., 2003).

Many microorganisms acquire the ability to transfer the electrons resulting from the metabolism of organic matters to the anode. Marine sediment, soil, wastewater, fresh water sediment and triggered mud are all rich sources for these microorganisms (Niessen et al., 2006 ; Zhang et al., 2006).

Geobacter belongs to dissimilatory metal reducing microorganisms, which produce biologically useful energy in the form of ATP. During the dissimilatory reduction of metal oxides under anaerobic conditions in soils and sediments. The electrons are translocated to the ultimate electron acceptor such as Fe_2O_3 principally by a direct contact of mineral oxides and the metal reducing microorganisms (Lovely, 2004; Vargas, 1998).

Shewanella oneidensis MR-1, a DMRB strain, was selected as a model organism because it establishes great metabolic litheness (Myers and Nealson, 1988), has its genome ascertained (Heidelberg, Paulsen et al. 2002), and is genetically accessible with regard to both generating and complementing mutants (Gorby et al., 2006). Strain MR-1 was initially studied because of its ability to diminish metal oxides counting manganese and iron oxides (Myers and Nealson, 1988). The mechanisms responsible for metal oxide reduction are not fully illuminated, but it is clear that a number of c-type cytochromes and joined proteins are essential (Beliaev et al., 2005; Gorby et al., 2006).

3. OBJECTIVES

Alternative sources of energies are the need of the hour. Microbial fuel cell is a very promising technique to generate energy in the form of electricity from waste biomass . All the microbes are not equally efficient in generating potential difference. The mechanisms involved with electron transfer to microbial fuel cell (MFC) electrodes are almost same in all the bacteria. Keeping this in mind the present study aimed to fulfil the following objectives:

1. To study the potential of four bacterial isolates to generate potential difference i.e. voltage.
2. To study the effect of strong salts like NaCl and KCl in electron conduction through agarose salt bridge.
3. To study the effect of silver nanoparticles incorporated salt bridge in electron conduction to cathodic chamber.
4. To study the effect of various concentration of dextrose on voltage production.
5. To study the potential of four isolates when taken in all possible combinations to generate potential difference.

4. MATERIALS AND METHODS

4.1. MEDIA AND CHEMICALS

Nutrient Agar Media

Peptone- 5 g/l, NaCl -5g/l, Beef extract – 3g/l, Distill water – 1L, pH-7.2- 7.4, Agar-15g

Luria Bertani Broth

Caesin enzymic hydrolysate – 10 g/l, yeast extract – 5g/l, Nacl -10g/l

Minimal Broth medium

K_2HPO_4 – 7g/ l, KH_2PO_4 – 2 g/l, sodium citrate – 0.50 g/l, $MgSO_4$ - 0.10 g/l, $Al_2(SO_4)_3$ – 1.0g /l]

Catholyte

Phosphate buffer (50 mM KH_2PO_4; pH 7.5)

Mediator

Methylene blue (**$C_{16}H_{18}N_3SCl$) – 10 ml.**

Nanoparticle synthesis

0.002M sodium borohydride ($NaBH_4$)- 30ml, 0.001 M silver nitrate ($AgNO_3$) – 2ml

Salt Bridge

1M , 2M, 3M KCl- 20ml, 1M , 2M, 3M NaCl, 3% agarose.

Sugar Stock (Carbon Source)

20% Dextrose stock was prepared in autoclaved Distill water, syringe filtered prior to use.

4.2. BACTERIAL ISOLATES

Four bacterial isolates (*Alcaligens, Stenotrophomonas, Pseudomonas, Penibacillus*) previously isolated from Paradeep, Chilika salt water lake, Rushkuliya and Bhitarakaneeka mangrove forest and stored at laboratory were used for MFC studies.

4.3. CULTURE CONDITIONS

The isolates were stored as stab at 4^{o}C. The culture was retrieved by streaking on nutrient agar plates and incubated at 37^{o}C. For MFC operation 2-3 isolated colonies were inoculated in 20 ml of LB broth and incubated at 37^{o}C at 160 rpm in shaking condtions.

4.4. MFC DESIGN AND COMPONENT

Electrode

Carbon electrode (Graphite) of dimension 15cm×2cm were used at both the ends of cathode and anode and tightly fixed with the containers containing medium, culture and buffer.

Cathodic chamber

The cathode chamber of the MFC was made up of 2 liter plastic bottles filled with aerated phosphate buffer (50 mM K_2HPO_4; pH 7.5) as catholyte.

Anodic Chamber

2 liter plastic pearlpet bottles were used for this purpose. The bottles were surface sterilized by washing with 70% ethyl alcohol and 1% $HgCl_2$ solution followed by UV exposure for 15 minutes. Then the autoclaved minimal medium broth was filled in it. Methlene blue and syringe filter sterilized dextrose solution was added to it and the caps containing electrodes were tightly fixed to it. Then 20 ml of previously enriched culture of bacteria (Luria Bertani broth medium for 12 hrs) was added.

Salt bridge

The salt bridge was prepared by dissolving 3% agarose in 1M KCl (Figure-3). The mixture was boiled for 2 minutes and casted in the PVC pipes (dimension 10 × 3 cm) in aseptic condition .The salt bridge was properly sealed and kept in refrigerator for proper settling. The molar concentration of KCl was changed from 1M to 2M for making the salt bridge. The percentage of agarose added and other components were the same. Again NaCl was used in place of KCl for making the salt bridge. The concentration of NaCl taken was 1M, 2M and 3M. Two holes were made in the lower side of bottles for the insertion of salt bridge. The lead was sealed with Mseal.

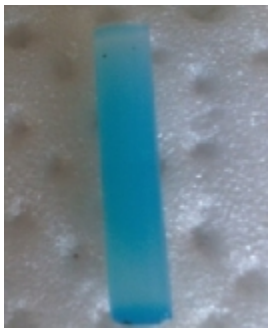

Figure -3.Image of salt bridge

4.5. Preparation of Silver Nanoparticles

0.002M sodium borohydride ($NaBH_4$) solution was prepared.30 ml of $NaBH_4$ was added to an conical flask. A magnetic stir bar was added and the flask was placed on an ice bath on a stir plate. The liquid was stirred and cooled for about 20 minutes.

Then 2 mL of 0.001 M silver nitrate ($AgNO_3$) was driiped into the stirring $NaBH_4$ solution at approximately 1 drop per second. Stirring was stopped immediately after the addition of $AgNO_3$. 1M KCl was added in the colloidal sol of nanoparticles in which 3% agarose was added into it. The mixture was boiled for 2 to 3 min and then casted into the PVC Pipe.

4.6. Mediator

Methylene blue is a redox indicators act as electron shuttles that are reduced by microorganisms and oxidized by the MFC electrodes thereby transporting the electrons produced via biological metabolism to the electrodes in a fuel cell (Bennetto et al., 1983).

4.7. Circuit Assembly

Two chambers were internally connected by salt bridge and externally the circuit was connected with copper wires which were joined to the two electrodes at its two ends and to the multimeter by another two ends.

4.8. Measurement of potential difference and current

The potential difference generated by the Fuel Cell was measured by using multimeter from MASTECH (figure-4) (Model No- M830BZ).

Figure -4. A multimeter

4.9. Different formulations of salt bridge containing nanoparticles

1M, 2M, 3M of KCl was added in 15 ml of the nanoparticle colloidal solutions in separate conical flask. Then 3% agarose was added in all the three concentrations taken above. They were boiled for 2 min and the casted in PVC pipes. Similarly 1,2,3M NaCl was added in 15 ml nanoparticles colloidal solutions along with 3 % agarose and casted.

4.10. Combinations of microorganisms used-:

Bacterial strain combinations
Alkaligens + *Stanotrophomonas*
Stanotrophomonas + *Pseudomonas*
Pseudomonas + *Paenibaccilus*
Paenibaccilus + *Alkaligens*
Alkaligens + *Pseudomonas*
Stanotrophomonas + *Paenibaccilus*
Alkaligens + *Stanotrophomonas* + *Pseudomonas* + *Paenibaccilus*

4.11. MFC operations

All the components of MFC are connected i.e. via salt bridge internally and with externally with wires to the mutimeter as it can be seen in figure -5.

The 2-3 isolated colonies were aseptically transferred in 20 ml LB broth and incubated at 37°C at 160 rpm for 12 hours. The bottles were surface sterilized prior to operation of MFC by 70% alcohol and 1% $HgCl_2$. Then it was exposed to UV radiation for 20 minutes.the salt bridge was sealed inside the holes with mseal in aseptic conditions. 2 liters of Minimal media broth was added in the anodic chamber. The 20 ml overnight culture along with 10 ml mediator mediator methylene blue was added. The MFC wad operated at room temperature i.e 25°C. The MFC set up was kept at static conditions. The varied carbohydrate concentration was one by one tested along with all the four isolates for their ability to generate potential difference. The MFC was run up to 12 hrs and the voltage was recorded at every 1 hr interval in all the cases.

Figure- 5. Complete setup of MFC.

5. RESULTS

5.1. Effect of Strong Salts like sodium chloride and potassium chloride: In the experiment conducted by employing KCl and NaCl based salt bridge, the maximum current produced was 262 µA and 122 µA respectively

Molar Concentration of Salt: The concentration of salt in salt bridge is highly desicive in transporting the hydrogen ions. Maximum current of 262 µA (Figure-7) was obtained in 1M concentration of KCl and 1M NaCl in Salt Bridge (Table-1).

TABLE-1Maximum current generated at different concentrations of KCl salt

Salt concentration (KCl)	**Maximum current generated (µA)**
1M	262
2M	120
3M	111

The maximum current of 122 µA was obtained with 1M NaCl (TABLE-2). Nacl was not that efficient like KCl as the current generated was almost half of that generated with KCl (Figure-8).

TABLE-2 Maximum current generated at different concentrations of NaCl salt

Salt concentration (NaCl)	Maximum current generated (µA)
1M	122
2M	90
3M	76

5.2. Effects of Nanoparticles on current generation

The current generation was increased when silver nanoparticles were incorporated along with 1M KCl in the salt bridge (TABLE-3). The silver nanoparticles help in better conduction of electrons through Salt bridge.

TABLE-3. Effect of nanoparticles on voltage generation

Silver nanoparticles + KCl	Maximum current generated (µA)
1M	343
2M	220
3M	130

A graph was plotted to compare the current generated at different concentrations of KCl and NaCl which shows that Silver nanoparticles incorporated salt bridge was most efficient in electrons transportation via saltbridge (Figure-6).

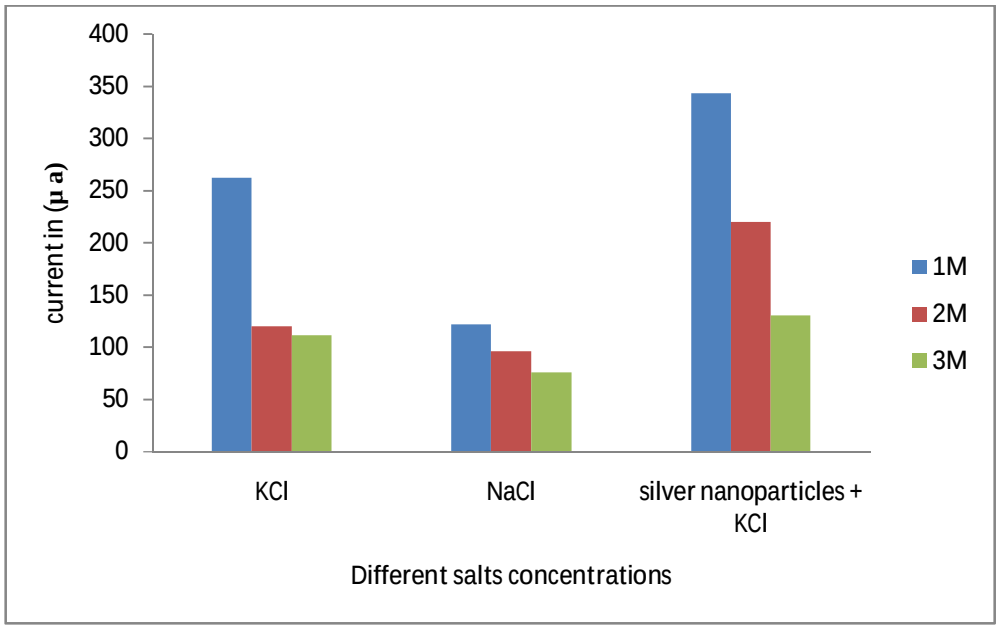

Fig-6. Graph representing current generated in various molar concentration of KCl, NaCl and silvernanoparticles + KCl

The organisms used for studying the effect of Salt concentrations were *Pseudomonas, Penibaccilus, Alcaligens* and *Stenotrophomons.* The maximum potential difference was generated with *Penibaccilus* in the salt bridge fabricated with 1M KCl and silver nanoparticles (Figure-9)

Fig-7. Maximum current (µA) generated with 1 M KCl

Fig-8 .Maximum current (µA) generated with 1M NaCl

Fig-9. Maximum current (µA) generated with 1M KCl + silver nanoparticles

5.3. Effect of increasing carbohydrate concentration

The carbohydrate source used was dextrose. Different concentrations of carbohydrate solutions were made and filter sterilized by syringe filter method. The concentrations used were 5g / l, 6 g/ l ,7g/l , 8g/l, and 10g /l (Table-4). It was found that maximum voltage was generated when dextrose was added in concentration of 7g/ l (Figure-10)

TABLE-4.Voltage generated by four strains at different carbohydrate concentrations

Concentration of dextrose solution used in g /l	Maximum voltage generated in Mv			
	Alcaligens	*Stenotrophomonas*	*Pseudomonas*	*Penibaccilus*
5	190	221	523	792
6	211	343	810	970
7	295	365	1010	1033
8	213	326	1001	1023
10	217	311	977	1013

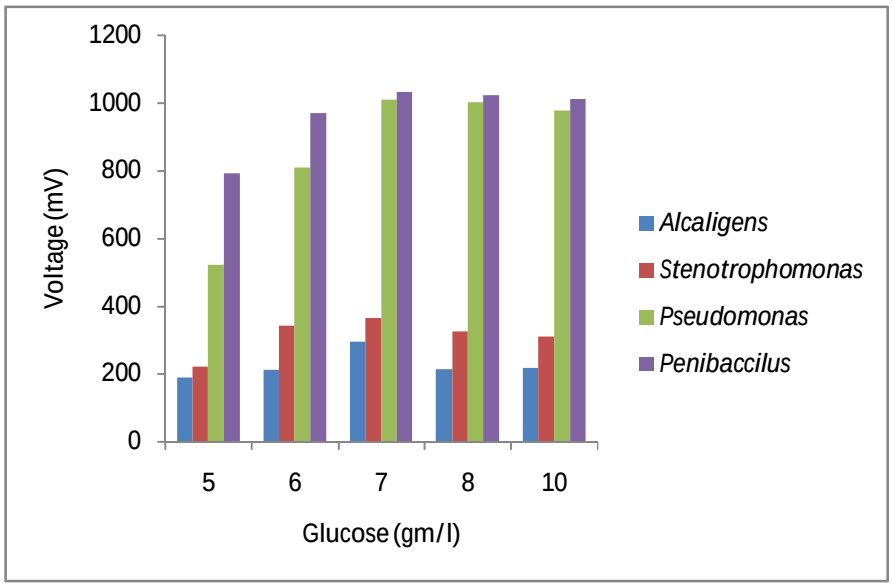

Figure-10. Graph showing voltage generated at different concentration of glucose added.

At dextrose concentration 7g/l the maximum voltage was generated by *Penibaccilus* that is 1033 mV (Figure-11) followed by *Pseudomonas* 1010 mV (figure-12), *Stenotrophomonas* 365mV (Figure-13) and *Alcaligens* 295 mV(Figure-14).

Fig-11. Maximum voltage generated by *Penibaccilus* at carbohydrate concentration 7 g/l

Fig-12. Maximum voltage generated at carbohydrate concentration 7 g/l by *Pseudomonas*.

Fig-13. Maximum voltage generated by *Stenotrophomonas* at carbohydrate concentration 7 g/l

Fig- 14.Maximum voltage generated by *Alcaligens* at carbohydrate concentration 7 g/l

5.4. Voltage generation potential of different organisms used as pure culture

The voltage generation was recorded at the interval of 1 hour up to 12 hours for all the four strains individually in presence of mediator. There was a definite increase in the voltage with the increase in time as we can see from table - 5.

TABLE- 5. Voltage generated by four strains when methylene blue mediator was used.

Bacterial strain	Voltage generated(mV) At zero hrs	Voltage generated(mV) After 1 hr	Voltage generated(mV) After 3 hrs	Voltage generated(mV) After 6 hrs
Alcaligens	60	130	253	346
Stenotrophomonas	85	175	293	428
Pseudomonas	110	200	373	691
Penibaccilus	122	353	423	718

It was observed that among the four strains used *Penibaccilus* have the maximum potential to generate electricity. Figure-15 shows the voltage generated after six hours by all the four strains. *Penibaccilus* shows the maximum voltage of 718 mV by *Penibaccilus* (figure-16) followed by *Pseudomonas* which generated potential difference of 691 mV after six hours (figure- 17).

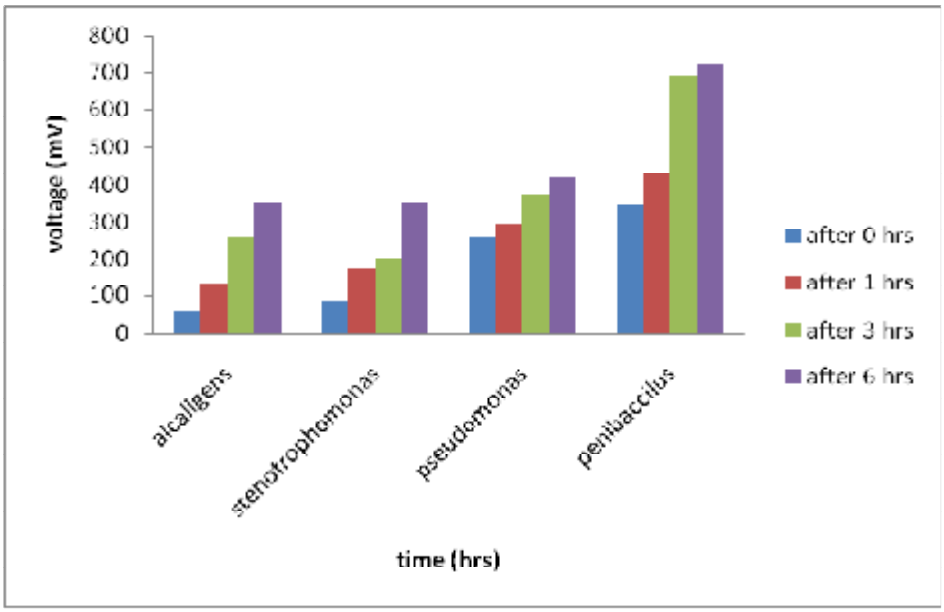

Figure-15. Graph showing the potential of individual organism when used in pure culture.

Figure-16. Potential difference generated by *Penibaccilus* after 6 hrs.

Figure-17. Potential difference generated by *Pseudomonas* after 6 hrs.

The capacity to generate potential difference was very low in case of *Alcaligens* and *Stenotrophomonas*. After six hours the potential difference generated was 346 (Figure -18) and 428 mV (Figure -19).

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Fig-18. Potential difference generated by *Stenotrophomonas* after 6 hrs.

Fig-19. Potential difference generated by *Alcaligens* after 6 hrs.

5.5. Voltage generation potential of different organisms used in different combinations.

In literature it has been found that organism shows good production of electricity when they are in consortium rather than when pure culture is used. The bacterial strains were than taken in combination of two i.e. consortium of two bacterial strains was made and potential difference generated was observed. Seven possible combinations were made with four bacterial strains taken for my experiment and their ability to generate potential difference was observed at 1 hr interval upto 6 hrs (Figure -20). The concentration of carbohydrate source i.e dextrose solution added was 7 g/ l in all the cases. It was observed that the combination of *Penibaccilus* and *Pseudomanas* shows maximum power generation (Figure -21). After 6 hours the voltage generated was 870 mV which increases after 12 hrs to 1039 mV (Figure-18). It was the maximum voltage generated among the all the seven possible combinations (table-5). The consortium of all the four bacterial isolates were not that efficient in producing electricity as they were able to generate only 295 mV (figure-21) potential difference after 6 hours of inoculation and after that potential difference reading started to fall rapidly coming to zero after 9^{th} hour.

TABLE- 6 Potential difference generated when organisms were taken in consortium

Bacterial strains Combinations	Voltage generated (mV). At zero hrs	Voltage generated (mV).After 1 hr	Voltage generated (mV).After 3 hrs	Voltage generated (mV).After 6 hrs
Alkaligens + *Stanotrophomonas*	90	122	141	222
Stanotrophomonas + *Pseudomonas*	110	160	370	423
Pseudomonas + *Paenibaccilus*	170	423	678	866
Paenibaccilus + *Alkaligens*	99	140	390	568
Alkaligens + *Pseudomonas*	87	112	290	396
Stanotrophomonas + *Paenibaccilus*	102	222	429	629
Alkaligens + *Stanotrophomonas*+ *Pseudomonas* + *Paenibaccilus*	112	370	240	295

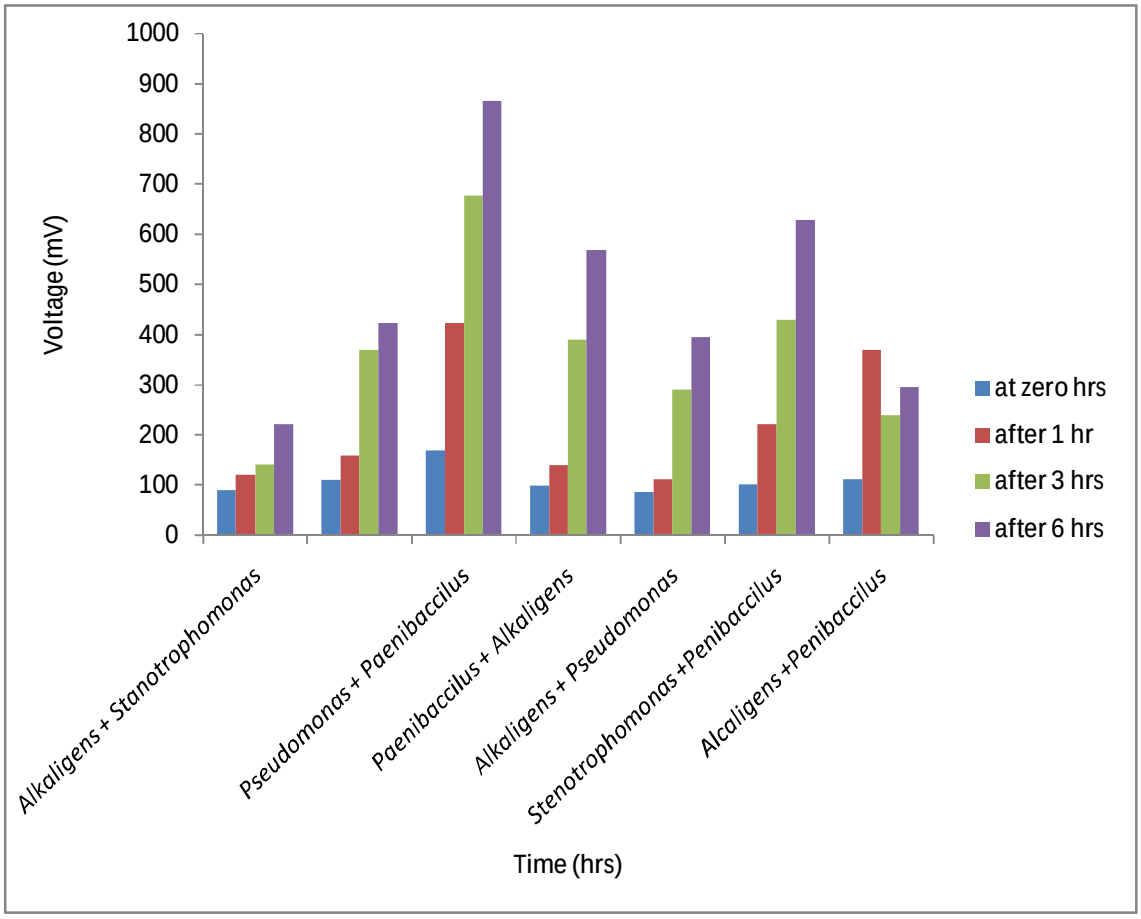

Fig-20. Graph showing potential difference generated by different combinations of bacterial isolates taken for the experiment.

Figure-21. Voltage generated by *Penibaccilus* and *Pseudomonas* consortium after 6 hours .

Fig-22. Maximum potential difference of 1039 mV generated by consortium of *Penibaccilus* and *Pseudomonas* after 20 hrs of inoculation.

Fig-23. Maximum potential difference of 295 mV generated by consortium of *Alcaligens, Stenotrophomonas, Pseudomonas* and *Penibaccilus.*

6. DISCUSSION

Microbial fuel cell is based upon the basic principle in which biochemical energy is converted into electrical energy. Consumption of organic substrate (e.g. sucrose) by microorganism in aerobic condition produce CO_2 and H_2O. If the terminal electron acceptor oxygen is replaced by mediator then the electrons will be trapped by mediater, which will get reduced and transpor to e^- to the electrode at anodic chamber .However when oxygen is not present they produce carbon dioxide, protons and electrons as described below

$$C_{12}H_{22}O_{11} + 13H_2O \rightarrow 12\ CO_2 + 48H^+ + 48e^-$$

It was first reported by M.C.Potter in the year 1910 with *E. coli.*

For present study 2L pearlpet bottle was used for making the anodic and Cathodic chamber. In Cathodic chamber minimal medium broth was used as the electrolytic solution where as Phosphate buffer was used as catholyte. Methelene blue and glucose as mediator and carbon source respectively in the anodic compartment. Methylene blue crosses the outer cell lipid membranes and plasma wall; it then begins to liberate electrons from the electron transport chain that would normally be taken up by oxygen or other intermediates. The reduced mediator carries electrons from the cell to the electrode. Here the mediator is oxidized as it deposits the electrons. These then flow across the wire to the second electrode, which acts as an electron sink. The maximum voltage of 300mV with *E. coli* was obtained by M.C.Potter.

Strong salts KCl and NaCl in 1M, 2M and 3M concentrations were used for fabricating salt bridge. The results obtained were comparable with the previous results. KCl was efficient most in transporting H^+ ions in the cathodic chamber (Muralidharan et al., 2011).The silver nanoparticles incorporated salt bridge was also tested for its efficacy to transport H^+ ions and it was observed that initially the voltage rise rapidly but soon the voltage starts falling down.

The carbon source used was glucose solution (Scott and Murano, 2007). The optimum concentration was found to be 7g/l for all the four isolated strains. An early paper by Rao et al. (1976) describes much of the initial work focussed towards developing glucose powered fuel cells for use within heart pacemakers.

The preferred organisms for MFC operations are metal reducing, anodophillic and flagellated microorganisms. *Geobacter* species are of interest because of their novel electron transfer capabilities, the ability to transfer electrons outside the cell and transport these electrons over long distances via conductive filaments known as microbial nanowires (Booki et al., 2005). Several organisms that are known to produce fermentation products and belong to the genus *Clostridium, Alcaligenes, Enterococcus,* have been used for MFCs operations (Rabaey et al., 2004).Four different bacterial isolates were tested for efficacy in producing potential difference. The four marine bacterial isolates belonging to genus *Penibaccilus, Pseudomonas, Stenotrophomonas* and *Alcaligens* were use as electron donor. They were studies as single cell MFCs and in all possible combination. Marine bacteria develop biofilms on the MFC electrodes, allowing considerable conversion capacity and opportunities for extracellular electron transfer (EET) (Suzanne et al., 2010). There is fewer information about bacteria belong to genus *Penibacilus* and its application in MFC which gave the maximum potential difference i.e. voltage generation of 1033 mV followed by *Pseudomonas* which generate the voltage of 1010 mV. Salt bridge was use for proton transportation into the cathode as it is very cheap compared to nafion membranes and equally efficient. The consortium of penibaccilus and Pseudomonas produced maximum voltage of 1039 mV.

The maximum potential difference reported by Cahyani and Gerard in the year 2008 for *Pseudomonas* is 0.2 V but it is with nafion membrane which is very expensive and comparatively the result obtained here is 1010 mV with simple salt bridge.

MFC have wide application in Biohydrogen production via Bioelectrolysis, Wastewater Treatment and Cathodic Denitrification, Bioremediation, Biosensors, In-situ Power Source for Remote Areas (Kim et al., 2008).

7. CONCLUSION

Microorganisms that can combine the oxidation of organic biomass to electron transfer to electrodes put forward the self-sufficient systems that can successfully convert waste organic matter and reusable biomass into electricity. Oxidation of these newly rigid sources of organic carbon does not supply net carbon dioxide to the environment and unlike hydrogen fuel cells, there is no requirement for wide pre-handing out of the fuel or for costly catalysts. With the suitable optimization, microbial fuel cells might be able to power an extensive collection of broadly used procedure. For example, there is current research on the future for powering self-feeding robots and even vehicles in this way. However, considerable optimization of microbial fuel cells will be required for most applications. Further investigations into the physiology and ecology of microbes that transfer electrons to electrodes are essential to carry out these optimizations in a rational manner.

Marine bacteria develop biofilms on the MFC electrodes, allowing considerable conversion capacity and opportunities for extracellular electron transfer (EET). *Penibaccilus* generated the maximum potential difference i.e. voltage generation of 1033 mV followed by *Pseudomonas* which generate the voltage of 1010 mV in single cell MFC. The consortium of *Penibaccilus* and *Pseudomonas* also shows very high production of potential difference of 1039 mV.

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